CHAPTER 5

DISCUSSION

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5.1 Effect of Carbon Dioxide supplementation on *Chlorella* production

There was marked increase in algal yield in the ponds supplemented with CO_2. The increase was however more distinct in the first two batches where the total carbon content in the RE was lower. Azov *et al.* (1982) also observed that at high initial carbon concentration in wastewater CO_2 addition had no effect. High CO_2 concentration in waste can also be attributed to aerobic degradation of organic substances through bacterial respiration.

Rubber effluent is a suitable substrate for algal growth although it has proven to be carbon limited (Geetha, 1992; Chui, 1994). Azov *et al.* (1982) suggested that carbon limitation of biomass production in a high rate oxidation pond is due to low free CO_2 concentration. However productivity in an outdoor system is also dependent on abiotic factors like the quality and quantity of light, temperature, nutrient concentration, oxygen, carbon dioxide and agitation (Becker, 1982; Borowitzka 1991). Rainfall during the growth period contributes to some dilution of pond content, although the pond in this study was covered.

Algal blooms are a natural occurrence in aerobic ponds treating rubber efluent. This was observed by Phang (1990) and Ho (1974). Kulkarni (1972) and Phang (1990) found RE to be a suitable medium for algal growth as it is rich in carbon, nitrogen and phosphorous. Geetha *et al*. (1992) showed good growth of *C. vulgaris* in RE. Chui (1994) obtained specific growth rates(μ) of *C. vulgaris* in RE ranging between 1.11 to 2.98 day^{-1} while μ in BBM ranged between 0.37 to 0.5 day^{-1}. In the present study algal growth in RE (μ = 0.36 day^{-1}) was superior to that in BBM (μ = 0.16 day^{-1}). (Table 4.2)

Stoichiometry of algal production is given by the following equation (cited in Shelef *et al*., 1976)

$$122\ CO_2 + 16NH_4^+ + PO_4^{3-} + 58H_2O \longrightarrow C_{122}H_{179}O_{44}N_{16}P + 131O_2 + H^+$$

Therefore waste grown algae are composed of 56.3%C, 8.6%N and 1.2%P. The "Algal Growth Potential" can be calculated based on the assumption that 1.0g C yields 1.91g algal biomass, 1.0g N yields 10.87g algal biomass and 1.0g P yields 76.92g algal biomass (Azov *et al*. 1977; Edwards *et al*., 1980). This model is good for estimation of biomass for a given concentration of nutrient when all other factors are in excess, and not limiting.

Table 5.1 compares the maximum yield based on dry weight to the Algal Growth Potential (AGP). The yield obtained in all batches were lower than the possible yield obtainable with initial concentrations of carbon, nitrogen and phosphorous present. Other factors such as the solar radiation may be limiting or that at high cell densities, light attenuation was great, therefore limiting light availability to the cultures.

Azov *et al.* (1982) suggested the use of BGP (Biomass Growth Potential) instead which is defined as :- 1g of C yields 2g of biomass, 1g of N yields 10 g of biomass and 1g of P yields 100g of biomass. BGP is useful when algal growth is limited by a certain nutrient, which in this study, is carbon.

The CO_2 aerated pond showed better growth in batches I, II and III. In Batch III although CO_2 aeration time was increased to 40 minutes per hour the algal growth was not equally increased. This may be because in Batch III the total solids and the total suspended solids content with RE were higher, thereby resulting in a lower growth rate. Lower light penetration probably also occured, which led to lower utilization of CO_2.

TABLE 5.1: Algal Growth Potential (AGP) and the observed yield in the HRAP

BATCH	TOTAL CARBON			AMMONIACAL NITROGEN			ORTHOPHOSPHATE			OBSERVED
	Inf.	%Rem.	AGP	Inf.	%Rem.	AGP	Inf.	%Rem.	AGP	MAX. YIELD
IC	728.63	0.50	1384.39	124.70	60.40	1355.49	10.04	77.80	772.28	* 207.00
ICO	728.25	0.30	1383.68	172.97	17.10	1880.18	13.71	47.50	1054.57	* 293.00
IIC	476.25	6.60	904.88	174.39	42.10	1895.62	15.12	53.30	1163.03	# 285.00
IICO	476.25	5.50	904.88	174.39	44.40	1895.62	15.12	51.70	1163.03	* 377.90
IIIC	1573.13	5.30	2988.94	407.07	5.30	4424.85	55.23	54.00	4248.29	756.00
IIICO	1573.13	3.30	2988.94	407.75	10.00	4432.24	62.29	72.10	4791.35	840.00
IVM	1286.25	19.30	2443.88	283.80	6.40	3084.91	22.70	81.80	1746.08	1324.80
IVCO	1421.25	2.10	2700.38	299.69	13.40	3257.63	22.53	89.70	1733.01	870.00
VC	1308.75	4.10	2486.63	367.73	47.60	3997.23	25.68	78.70	1975.31	@ 540.00
VMCO	1395.00	44.20	2650.50	408.00	35.50	4434.96	30.25	73.00	2326.83	@ 1367.00

Observed Max. Yield = Max. chll−a X 67

Inf. = influent

% Rem. = Remainder

all units are in mg/l

* values for day 3

value fot day 2

@ final day values

Syamala *et al*. reported a 76% increase in CO_2 enriched autotrophic axenic cultures compared to the control and also beter yield of biomass in molasses cultures of *Scenedesmus acutus* supplemented with CO_2 and molasses. In this study the increase in algal biomass over the control based on chlorophyll-a concentration, is 29% for Batch I, 24.4% for Batch II and 10% for Batch III. The decreased improvement in growth in Batch III is due to the poor effluent quality, in this particular batch due to the high total solids and total suspended solids content.

In general CO_2 supplementation contributed to better productivity of *Chlorella vulgaris* (Table 5.2) and hence is expected to improve biomass production in the High Rate Algal Ponds.

5.2 Effect of supplementation of CO_2 together with molasses on *Chlorella* production

In the current study enrichment of cultures with CO_2 enhanced growth rate (μ = 0.64) in the laboratory experiments, compared to the control (μ = 0.44), while the cultures supplemented with molasses gave even better growth rate (μ = 0.74).

In Batch IV the supplementation with molasses improved

TABLE 5.2 : **Gross biomass productivity obtained under batch culture conditions**

	Max. Algal Conc.	PRODUCTION		Retention Time	PRODUCTIVITY	
		Area	Volume		Area	Volume
POND	mgL^{-1}	mgm^{-2}	mgm^{-3}	day	$mgm^{-2}d^{-1}$	$mgm^{-3}d^{-1}$
I C	85.10	22.39	149.30	1.50	56.73	99.53
I CO	152.10	40.03	266.84	1.50	101.40	177.89
II C	386.50	101.71	678.07	1.50	257.67	452.05
II CO	507.90	133.66	891.05	1.50	338.60	594.04
III C	464.30	122.18	814.56	4.00	116.08	203.64
III CO	743.70	195.71	1304.74	4.00	185.93	326.18
IV M	705.50	185.66	1237.72	2.50	282.20	495.09
IV CO	602.30	158.50	1056.67	2.50	240.92	422.67
V C	249.20	65.58	437.19	4.00	62.30	109.30
V MCO	542.00	142.63	950.88	4.00	135.50	237.72

algal production as much as 34.5% compared to the CO_2 supplemented pond, indicating that *Chlorella vulgaris* 001 is heterotrophic and both autotrophic and heterotrophic growth combined gave good algal production. In Batch V the improvement in growth in the ponds supplemented with both molasses and CO_2 had an improved growth of as much as 60.5%, indicating that *Chlorella vulgaris* 001 is able to utilise both organic and inorganic carbon that are present in the same growth medium.

5.3 Treatment Efficiency of the HRAP

The basic concept of a HRAP as a waste treatment system is to utilize the ability of the algae to incorporate the nutrients available in the waste for biomass generation. This results in two outputs from the system namely "clean water" and a useful biomass.

Freshwater algae have been used in several waste water systems from tapioca starch waste water (Tanticharoen *et al.* 1990) to municipal wastes or sewage (Shelef *et al.* 1980; Kawai 1984; Edwards *et al.* 1980). Craggs *et al.* (1994) have also used marine microalgae for nutrient removal in sewage effluent.

In this study, good COD removal (55.8% - 99.7%) was attained, giving COD values well below the DOE standards

(5 - 106 mg/l). Good COD removal also reflects the carbon limitation of RE for algal growth. For the ponds supplemented with molasses, however, the COD values were higher because molasses had a high dissolved COD content. The presence of some undegradable substances in molasses could be contributing to the remaining COD in the final effluent.

In general, the final effluent quality obtained complied with DOE standards for the parameters monitored (Table 5.3). Removal of NH_3-N and PO_4 was dependent upon the C:P and N:P ratio (Jones *et al.*, 1987). Low C:P ratio could reduce cellular uptake of phosphate. Therefore the increase of C:P ratio should increase removal of phosphate. In the present study in the ponds enriched with CO_2 the removal of phosphate as compared to the control was similar but in the ponds supplemented with molasses (organic carbon source) phosphorous removal was better compared to the control as the addition of molasses increased the C:N:P ratio. Przytocka-Jusiak *et al.* (1984) and Matusiak (1976) showed that nutrient removal efficiency is also related to (i) the contents of nutrient wastewater, (ii) the degree of nutrient utilization by algal growth and (iii) the nutrient concentration in algal biomass.

Table 5.3: Final effluent quality obtained in the HRAP

Pond	COD mg/l	NH_3-N mg/l	PO_4-P mg/l
IC	10.00	75.30	7.81
ICO	5.00	29.65	6.51
IIC	84.00	73.37	8.06
IICO	70.00	77.50	7.62
IIIC	224.00	21.70	29.82
IIICO	140.00	40.80	44.90
IVM	472.00	18.07	18.57
IVCO	52.00	40.01	20.22
VC	106.00	174.93	20.47
VMC	1092.00	144.76	22.09

Although NH_3-N has been found to be toxic (≥ 2 mM) to (Abeliovich, 1980) algal growth and photosynthesis (expressed by dissolved oxygen values) the concentrations in the rubber effluent did not inhibit the the growth of *Chlorella vulgaris* 001. *Chlorella vulgaris* 001 has the unique ability to grow in high NH_3-N levels.

NH_3-N can be incorporated into algal biomass and therefore discarded from the waste water or volatilised out of the waste water through high pH values and elevated temperature (Reeves, 1972). However in this study the pH values were only between 6.63 - 8.41 . This is not sufficient for "ammonia stripping".Therefore algal biomass was solely responsible for depletion of NH_3-N in the HRAP Batches.

Phosphate can be removed through incorporation into algal biomass. However in this case enhanced growth in the CO_2 ponds has not yielded better phosphate reductions as compared to molasses supplemented ponds. Chui(1994) found better phosphate reductions in molasses supplemented ponds.

Tredici *et al.* (1992) cultivated *Scenedesmus* sp. in secondary treated waste water and attained excellent removal of nitrogen and phosphorous in a 24h cycle. The efficiency of nitrogen incorporation varied from 40.6% to 49.5% while that of phosphorous was between 69.5% to 85.2%. The system treated 75L sewage m^{-2} day^{-1} therefore removing 1.0g N and 0.1g P per m^{-2}.

The HRAP system offers an economically feasible alternative to current treatment systems. It allows cost reduction of capital; recovery in the form of the algae biomass harvested which can be sold as animal protein supplement or as aquaculture feed.

The retention time for use in a continous system in the current study also varied from one batch to another but averaged around 6 days for the pond volume of 450 litres in an area of $4m^{-2}$. If the detention time in the pond is reduced treatment efficiency could be reduced and in a continous system the High Rate Pond would turn anaerobic

(Abeliovich, 1980).

The current study proves that treatment of rubber effluent using the High Rate Algal Pond system is promising in terms of effluent quality obtained. Kawai *et al.* (1984) felt that High Rate Photosynthyetic Pond is better because it produces better quality effluent discharge compared to activated sludge for treating sewage, while the biomass obtained can be commercialized to be a protein source, therefore making the HRAP treatment system economically viable.

5.4 General Performance of the HRAP

5.4.1 *Chlorella* Biomass Production (Table 5.2)

The biomass production of *Chlorella* was bettter in ponds supplemented with CO_2(40.03mgm^{-2} to 195.71mgm^{-2}) than with the controls(22.39mgm^{-2} to 122.18mgm^{-2}).

5.4.2 Biochemical Composition of *Chlorella* Biomass

There was no significant difference between biomass obtained from the CO_2 enriched ponds and the control ponds. The nutritional value of the algal biomass harvested is comparable to the animal feed requirement (Table 5.4). John (1975) showed that RE grown *Chlorella* is suitable as a feed supplement for most animals, especially fish. *Tricipidis leevi* and *Tilapia* are examples of freshwater fishes that grow well in stabilization ponds treating block rubber factory effluent and fed solely on the algae in the ponds. De Pauw and Persoone (1988) have shown that rotifers, brineshrimp, *Tilapia* species, carp and milkfish can be fed with health-food grade *Spirullina* species and *Chlorella* species. Studies by Becker (1978) show that 10% of algae (*Scenedesmus*) supplied in rat feed to be safe while maximum body weight was observed in rats fed with 15% algae. Kawai *et al*. (1984) found that feeding chicks between 29 - 49 days old with algae gave higher weight increase.

Torzillo *et al*. (1991) observed a net increase in carbohydrate synthesis in *Spirulina platensis* when grown in low biomass and high light irradiance. The carbohydrate synthesized during the day was only partially utilized for night protein synthesis.

Becker (1978) reported the amount of total protein in *Scenedesmus acutus* to be the highest in cultures supplied with both CO_2 and molasses, while there was no significant difference between CO_2 enriched cells and molasses enriched cells.

5.4.3 Statistical Analysis on correlation of algal growth in the HRAP with physical-chemical factors

Correlation analyses between the maximum algal biomass was carried out in two parts:-

i. with the initial chemical content of RE and the physical growth parameters i.e. solar radiation, daily sunshine hours and rain

ii. with final effluent quality

Correlation values obtained for part (i) is summarised below:-

	Max. Algal Biomass
Max. Algal Biomass	1
Solar Irradiance	0.674
Sunshine Hours	0.642
Raindays	−0.627
pH	0.760
COD	0.416
NH_3	0.533
PO_4	0.673

It is obvious from the values obtained that the important factors in final algal biomass content are the intial values of pH and phosphate while solar radiation and number of sunshine hours per day also have a great influence. The relationship between algal yield and the aforesaid parameters are shown by the following regression equations:-

Algal yield = 3.305pH - 2.75 ($r=0.7598$; $p < 0.05$)

Algal yield = 1.634 PO_4 + 2.57 ($r=0.6725$; $p < 0.1$)

Algal yield = 2.5773 solar radiation - 1.140 ($r=0.6753$; $p > 0.1$)

Algal yield = 2.366 sunshine hours + 1.177 ($r = 0.6416$; $p > 0.1$)

To investigate the influence of algal biomass on the treatment efficiency, correlation studies were carried out between the maximum algal biomass and the final treated effluent quality. Correlation results are given in table below:-

	Algal Yield	COD	PO_4-P	NH_3-N	pH
Algal Yield	1	0.387	0.641	−0.309	0.559
COD	0.387	1	0.231	0.314	−0.021
PO_4-P	0.641	0.231	1	−0.099	0.774
NH_3-N	−0.309	0.314	−0.099	1	−0.012
pH	0.559	−0.021	0.774	−0.012	1

Significant positive coorelation was obtained between the algal yield and the phosphate and pH. Significant coorelation was also obtained between phosphate and pH.

5.4.4 Mode of Nutrition in the HRAP

In Batch IV the pond supplemented with molasses showed better growth compared to the pond supplemented with CO_2 indicating that *Chlorella vulgaris* 001 is able to utilize organic carbon better than CO_2. Abeliovich & Weisman (1978) found that light is not limiting for algal growth in waste water because apart from autotrophy, the cells may adopt an efficient glucose transport system under hetetrophic conditions. In oxidation ponds, cells had common features of both photoautrophic and heterotrophic nutrition. They suggested that the main source of glucose is through the degradation of polysaccharides through microbial activity and hence 15% of algal cell carbon can be obtained this way. Abeliovich (1980) found that in a High Rate Oxidation Pond of 40cm deep, the compensation point for net photosynthesis is 2 - 5cm and therefore hetetrophic growth is responsible for good algal growth.

In Batch V the treatment pond showed the best growth of all compared to the control (Table 4.5). Batch V optimised the ability of the algae to utilise two different carbon sources to enhance the growth and biomass. Similar results were also obtained by Becker (1978) using *Scenedesmus acutus* supplemented with CO_2 and molasses. But the dissolved oxygen values obtained on day 2, 3 and 4 in the treatment pond showed that algal cells were more heterotrophic than autotrophic. It could also be because the oxygen was being utilized the moment it was released into the media by the bacteria present, thus contributing to lower D.O. readings.

5.4.5 Semidiurnal Changes

Semidiurnal studies were carried out for 12 light hours (0630 to 1830) and various physical parameteres besides cell count and chlorophyll-a were monitored.

5.4.5.1 Cell count and chll-a

Both chll-a and cell count are proportional to the growth of the algae in the HRAP. An increase in the cell number will indicate division of cells in the pond. This was in all studies during the logarithmic phase. Division of cells seem to occur mostly in the afternoons between 1430 and 1630. This is usually indicated by an incrase in cell number but no parallel increase in chll-a content. This was reflected in the decreased chll/cell ratio.

Cells from CO_2 enriched ponds had a higher chll-a:cell ratio compared to those from control ponds. However CO_2 + molasses enriched cells in Batch V had lower chll-a/cell ratio compared to the control indicating that in heterorophic or mixotrophic nutrition chll-a was not required in as high a concentration as in autotrophic growth.

5.4.5.2 DISSOLVED OXYGEN

Dissolved oxygen values are good indicators of algal photosynthesis. In mass culture of algae in waste water, the economical and practical use is the ability to contribute oxygen for biodegradation of organic matter (Shelef *et al.*, 1980). Dissolved oxygen values in all pond enriched with CO_2 alone were higher than the controls ponds (Batch I, II and III) or the molasses supplemented pond (Batch IV). In Batch V the oxygen produced during photosynthesis was probably being taken by the bacteria present in the pond for immediate aerobic consumption, causing lower dissolved oxygen values to be recorded.

5.4.5.3 pH

pH of the HRAP became more alkaline during the afternoon due to *Chlorella* photosynthesis. As CO_2 is consumed by the algae, the rise in pH can be shown by the following equilibrium (Richmond, 1986).

$$CO_3^{2-} \quad + \quad H_2O \quad \longrightarrow \quad CO_2 \quad + \quad 2OH^-$$

In algal mass cultures, a rise in pH shows that the CO_2 supply in the aqueous medium is sufficient to meet algal growth requirements (Kaplan *et al.*, 1986). When organic substances are consumed, the pH will decrease due to CO_2 production (Buhr & Miller, 1983). This was observed in the ponds supplemented with molasses in Batches IVM and VMC. In ponds supplemented with CO_2, pH values were slightly more acidic than the control ponds probably due to the presence of the carbonic acid that was not utilised by the algal biomass.

5.4.5.4 Temperature and Irradiance

These are two physical factors which are very important for algal growth. In a High Rate Algal Pond the algae are subjected to two different rhythms of light dark cycle (Vonshak & Guy, 1992). In the first regime is the movement of the algae to the surface and are exposed to full sunlight and then are subjected to darkness at the bottom of the pond, usually at a depth of 12 -15cm. The second regime is relatively slower and involves the daily change in solar radiation from sunrise to sunset.

In the current study, Batches I and II had low irradiance compared to the other batches. This must have contributed to the generally lower productivity in these two batches compared to the others. In general, biomass in terms of chll-a increased from morning to evening during the exponential growth phase, following the increase in irradiance. Similarly D.O. levels which indicate photosysthesis also followed the changes in irradiance over the day. Chll-a per cell appeared to be high in the early morning to early afternoon when irradiance was high but decreased towards evening with decreasing irradiance. This indicates the relationship between chlorophyl content and photosysthesis with chlorophyl content increasing to harvest the solar energy. Also increase in chlorophyl number due to cell division may contribute to lower chlorophyl content per cell towards the end of the day.

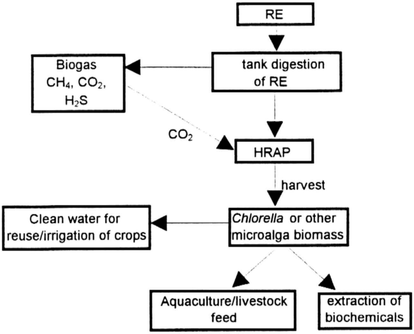
5.8 Potential use of HRAP in rubber industries

With growing population demands and possible future food and water shortages due to rapid urbanisation, it will be necesary to develop processes that can utilize readily available plant nutrients to produce food and recycle wastewater (McGarry & Tongkasame, 1971). The urgency to treat the 100 million litres of RE produced per day in Malaysia is there (Phang, 1987). In the current study rubber effluent has proven to be a good substrate for algal mass culture.

Chlorella has proven to be an excellent source of single cell protein with good nutritive qualities if the algal biomass is properly processed and fully digestible. Becker (1988) found that the best processing method for the breakage of rigid cell walls of *Chlorophyceae* is a short heat treatment (a few seconds at 110° to 120°C). The "Albazod" biomass - a term coined by (Soeder 1980) to described the product recovered form HRAP which consist of algal cells, bacteria, zooplankton and detritus and successfully tested as a protein source in feed for pigs, sheep, carp, *Tilapia* sp. and chickens.

Although in the current study productivity obtained under the HRAP were lower than of other reported values there is definately room for improvement. In the case of the first Japanese pilot plant the yield in the beginnig was only 3.5g/m^{-2}/day of *Chlorella* dry matter but increased to 16 - 17g/m^{-2}/day with improved technology (Soeder, 1980). Therefore under Malaysian Weather conditions there is definately a possibility of better productivity with the usage of continous systems and shorter retention time and more effecient CO_2 supplementation.

A proposed system for the mass culture of microalgae in rubber effluent is illustrated in the following page:-


RE
tank digestion of RE
Biogas CH_4, CO_2, H_2S
CO_2
HRAP
harvest
Chlorella or other microalga biomass
Clean water for reuse/irrigation of crops
Aquaculture/livestock feed
extraction of biochemicals